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A DIPLOID VARIETY IN THE CYSTOPTERIS FRAGILIS COMPLEX

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The mere perusal of floristic manuals gives no clue, as a rule, to the genetic significance of many "varieties" listed. For example, sometimes so-called "varieties" are just growth-forms, some of them even the result of injuries. On the contrary, many plants assigned this category possess phylogenetic importance, and in some cases have proved to be genetically isolated from other varieties of the same species. Observations on the ferns included in the *Cystopteris fragilis* complex over the last twenty years indicate more and more strongly that some of the "varieties" may have considerable phylogenetic significance—in fact there is substantial evidence that some may merit interpretation as species rather than as varieties. Excluding those which presently seem to have but little or no importance in forming the populations of northeastern North American *Cystopteris*, there are eight taxa which warrant study to determine their inter-relationships, namely *C. bulbifera* (L.) Bernh., *C. dickieana* Sim, *C. fragilis* (L.) Bernh. var. *fragilis*, *C. f.* var. *laurentiana* Weath., *C. f.* var. *mackayii* Lawson, *C. f.* var. *protrusa* Weath., *C. f.* var. *simulans* (Weath.) McGregor, and *C. f.* var. *tennesseensis* (Shaver) McGregor. Chromosome numbers of all of these forms have not been determined, but those which have been found in previous studies indicated that there might exist a diploid variety of *C. fragilis* with a chromosome number of $n = 42$, since the populations investigated have yielded numbers of $n = 84$ and 126, i.e., tetraploid and hexaploid respectively. The writers have now found such

a diploid variety for the first time, as will be herein reported. We have also studied some of the differences between the two common eastern American varieties, *mackayii* and *protrusa*, which are reputed to intergrade with each other. The evidence to be presented here indicates that they may not actually be able to intergrade, at least in the usual sense.

For making us the loan of the large Gray Herbarium series of these plants, we wish to acknowledge the kindness of Reed C. Rollins. We thank also Edgar T. Wherry and Conrad V. Morton for their assistance in various ways.

DIPLOID VARIETY OF C. FRAGILIS: A number of new chromosome data were presented for these ferns in an earlier article (Wagner, 1955). Two bulblet-producing, suspectedly hybrid, taxa, var. *simulans* and a plant that was tentatively identified as var. *tennesseensis* from the Lake Superior region, were found to possess chromosome complements of $n = 84$ and $n = 126$ respectively. Although the chromosome numbers heretofore known for the putative parents satisfied an allopolyploid hypothesis for the hexaploid "var. *tennesseensis*," the number known in "var. *simulans*" was inconsistent. If the latter were an allopolyploid, the fact that the lowest number thus far reported for *C. fragilis* or any of its known varieties was $n = 84$ did not conform. The possibility was indeed recognized, however, that there might exist now or might have existed at some time in the past more primitive populations of *C. fragilis* in which the chromosome number was diploid, i.e., $n = 42$, as it is in *C. bulbifera*.[1]

We wish to report living populations of *C. fragilis* found in late spring, 1955, that do show the predicted number of $n = 42$. During the course of his investigations of spores in the genus *Cystopteris*, one of us (Hagenah) noticed that unusually small-sized spores occurred in certain collections of this complex that were found in terrestrial situations growing on soil rather than epipetrically on rocks. We decided to make cytological

[1] Prediction of the possible existence, present or past, of diploid *C. fragilis* had already been anticipated by Manton (1950, pp. 121, 122) on different grounds, specifically that two chromosome numbers were known in British and European *C. fragilis*, $n = 84$ and 126. She wrote that "The relationship between numbers as different as 126 and 84 may not at first leap to the eye, yet we can hardly doubt that we are dealing with the upper members of a polyploid series, the lower ones of which are still to seek. If a form with a gametic number of 42 could be found we should have simple series of 42, 84, and 126. . . . It may be that the diploid is extinct."

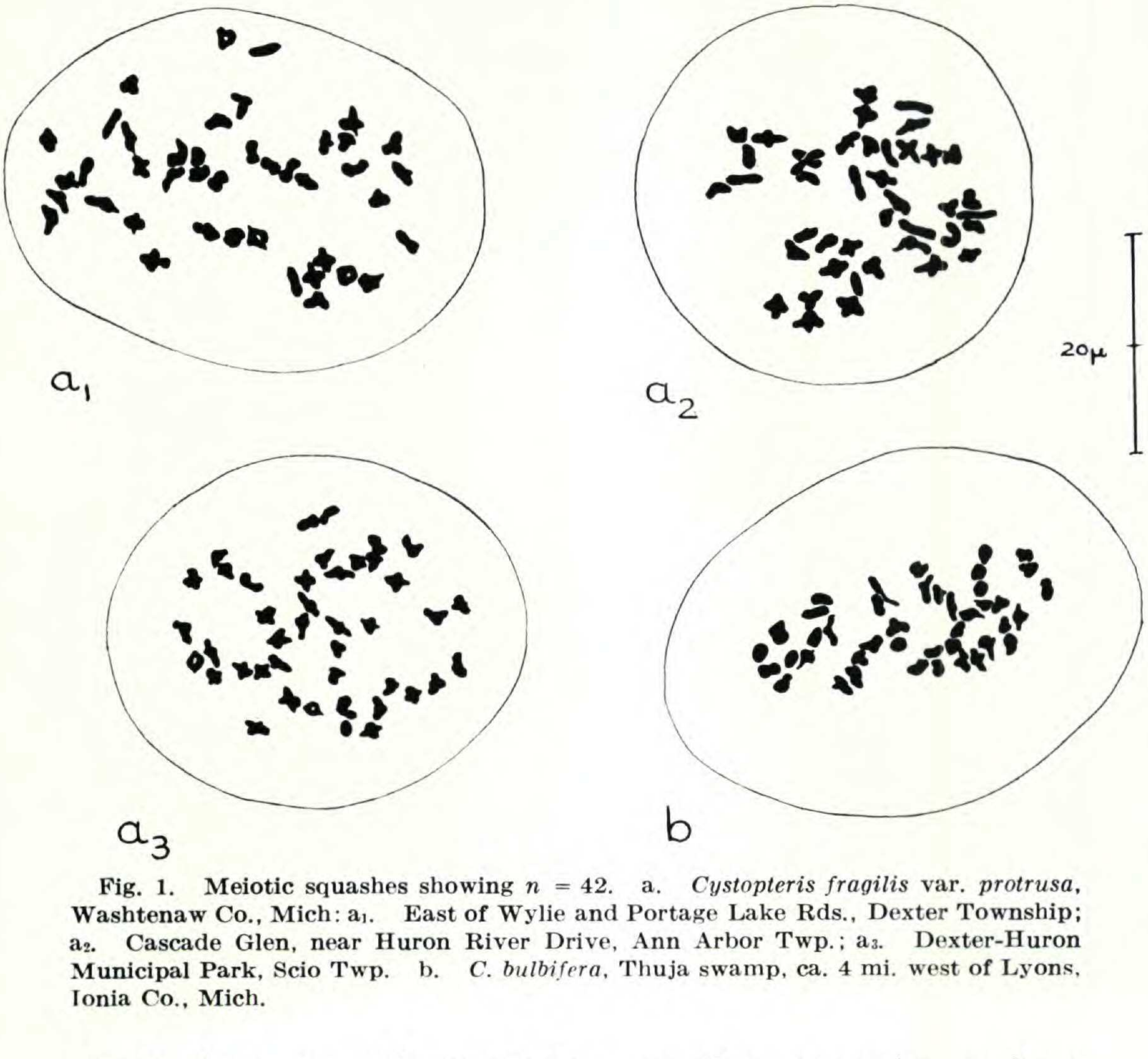

Fig. 1. Meiotic squashes showing $n = 42$. a. *Cystopteris fragilis* var. *protrusa*, Washtenaw Co., Mich: a_1. East of Wylie and Portage Lake Rds., Dexter Township; a_2. Cascade Glen, near Huron River Drive, Ann Arbor Twp.; a_3. Dexter-Huron Municipal Park, Scio Twp. b. *C. bulbifera*, Thuja swamp, ca. 4 mi. west of Lyons, Ionia Co., Mich.

observations on such populations, as they occur in southern Michigan, as soon as the unfolding crosiers of spring formed sori of sufficient size and development to make chromosome counts feasible. In late May, therefore, a preliminary field trip was made, and collections of fixed cytological material and voucher specimens were obtained from two terrestrial populations, one occurring in Lyndon Township, the other in Scio Township, in Washtenaw County, not far from Ann Arbor. The former collection was the first examined: it proved disappointing, the results showing clearly $n = 84$ at meiosis, the same number that had been found in all other North American populations thus far investigated with the exception of the glandular bulblet-bearing plant from the Great Lakes region. The matter was temporarily dropped at this point, and the

other collection was not examined for almost a week; but when observations were made of the latter, the sought-for number was obtained, and meiotic squashes demonstrated that the chromosome number was $n = 42$ (fig. 1).

An attempt was subsequently made to find out whether other terrestrial populations in the vicinity of Ann Arbor could be located of both types, the tetraploid and diploid, and it was especially attempted to determine whether the 84-numbered forms could be separated in the field from the 42-numbered ones. During the following month, three more populations were found of the diploid, and four more of the tetraploid. A list of them (plus two more records of *Cystopteris bulbifera*) is given in Table I.

VARIETIES MACKAYII AND PROTRUSA: It was soon obvious that the tetraploid terrestrial populations represent the taxon *C. fragilis* var. *mackayii* and the diploids var. *protrusa*. These two varieties are considered to be endemic to northeastern North American, and they were carefully described and distinguished by Weatherby in 1935, whose treatment has been followed by all subsequent authors. Both of these varieties differ from the typical one of the species in having indusia of considerably smaller size, the margins of which are nearly entire rather than deeply cleft. Weatherby (op. cit.) distinguished his var. *protrusa* from var. *mackayii* on the basis primarily of its rhizome and the shape of its pinnae. From the present studies it is now possible to give three additional contrasts—their chromosome numbers, the coloration of their stipes, and the laminar textures.

If there are real differences in the respective habitats of the populations of var. *mackayii* and var. *protrusa* that grow on the ground, it is not readily obvious in southern Michigan. It was hoped that there might be evident and consistent differences, but both of the taxa occur in rich woods of the same aspect, shaded by oaks, hickories, maples, and elms, and growing in more or less loamy soil. Poison ivy (*Rhus radicans*) is a frequent associate. Their herbaceous neighbors in the counties near Ann Arbor and Detroit commonly include *Laportea canadensis*, *Aster* spp., *Eupatorium* spp., and *Thalictrum dasycarpum* in the fall; and in the spring such rich-woods plants as *Ery-*

thronium americanum, *Dentaria laciniata*, and *Podophyllum peltatum*. In some soils the rhizomes grow nearly or quite superficially on the surface of the ground; in others they may be buried except for their apices. The only real difference we have been able to detect between the habitats of the two taxa is that var. *mackayii* apparently has a much greater amplitude in habitat tolerance than var. *protrusa*. Variety *mackayii* may occur perched up on old logs and woody roots. In the other variety such an occurrence has never been found: all of the populations thus far observed have been growing directly in soil at the level of the ground. Variety *mackayii* not only grows on logs and roots, but will also be found on more or less dry, and sometimes rather exposed, soil road-banks, and, as is well known, grows also readily on rock cliffs, directly in the crevices of the rock.

One of the sharpest distinctions observed in Michigan material of the two taxa involves the relative development of the pigmentation of the petioles. The varieties are illustrated side-by-side in Plate 1224. In var. *mackayii* the stipes are mostly shiny dark-chestnut in color, at least in the lower half. (Typical *C. fragilis* var. *fragilis* from Europe and N. America also commonly possesses this coloration). Very young leaves that were collected and pressed in the early springtime may lack this pigmentation, but only one herbarium specimen that appears to be typical var. *mackayii* in the Gray Herbarium series has been seen which appears to be devoid of the pigmentation in the mature state of the leaf ("mature" in the sense of having fully developed, sporiferous sori.) This is a collection made on the Tennessee River cliffs, opposite Knoxville, by Wherry and Benedict. However, in general, in large, well-developed fronds, the dark coloration of var. *mackayii* may extend even to the first pair of pinnae or sometimes even beyond. The smaller the frond, as a rule, the less extensive is the relative development of the coloration. Judging from the photograph of the Type of var. *mackayii* in the Gray Herbarium (Mill Brook, N. E. Dalhousie, Pictou, Nova Scotia, July 30, 1875, *A. H. Mackay*) the pigmentation of its petioles is the same. In var. *protrusa*, by contrast, the stipes are generally merely greenish or straw-colored. The dark coloration like that in var. *mackayii* occurs only at the very base of the stipe, running

at most only a centimeter up the petiole. Some individuals may have more extensive pigmentation of the stipes, but the color is pale-brownish at most. Also injuries sometimes appear to stimulate dark pigmentation to be produced in certain sectors of the petiole. But the rich, dark chestnut-brown of var. *mackayii* has not been observed.

The thicker texture of var. *mackayii* may be used to distinguish it from var. *protrusa* in the field, especially at the time of spore formation in the spring. On the herbarium sheet, moreover, the distinctions remain in this respect, although not so obviously: the pinnae of var. *mackayii* tend to have a hard "feel" as compared with those of specimens of var. *protrusa*. The leaves of living plants of var. *mackayii* stand much more rigidly upright, and they resist wilting after collection much more than do those of var. *protrusa*. Also the leaves of var. *mackayii* are considerably less fragile, and they do not tend to break at the base as do those of var. *protrusa*.

As is well shown in the typical examples in Plate 1224, the pinnae in var. *mackayii* are generally narrower and more remote than those of the other in fronds of equal size growing in comparable situations. The leaves of var. *protrusa*, on the contrary, are more ample and have a somewhat "feathery" appearance. The latter aspect is due to the fact that the basal secondary segments of var. *protrusa* tend to have definite petiolules, as pointed out by Weatherby in his original description. The lowest segments of var. *mackayii* are mostly more broadly based, and the segments themselves conform in general to Weatherby's description of being only shallowly toothed or lobed, and with a broad, rounded apex, and if more deeply lobed often lanceolate or oblong-lanceolate. The pinnules of var. *protrusa* are more sharply toothed or have more pointed lobes, and they are broader, being usually ovate-lanceolate to deltoid-ovate.

Table I. New Chromosome Counts in Cystopteris

	n
C. bulbifera (L.) Bernh.	
Thuja swamp, ca. 4 mi. W of Lyons, Ionia Co., Mich., June 7 (Wagner 8080)	42
Rocky woods, Grand Ledge, Eaton Co., Mich., June 7 (Wagner 8078)	42
C. fragilis var. *mackayii* Lawson	
Thuja swamp, south of Levering, Emmet Co., Mich., June 24 (Wagner 8087)	84

The rhizomes of the two entities are usually quite unlike, and most authors have emphasized this difference. Those of var. *mackayii* generally have very short internodes, the leaves being borne in juxtaposition, and those of var. *protrusa* have very long internodes, the leaves being, accordingly, very widely separated. In the latter taxon the shoot apex protrudes usually a couple of centimeters, at least, beyond the leaves. This condition does not show of course so well in the fall and winter, and it should be pointed out that when the leaves are not present the individual stems of the two entities can occasionally be confused. For example, certain individuals of soil-grown plants of var. *mackayii* may have long lateral branches, the internodes of which are as long as those of var. *protrusa* or nearly so. Weatherby has already noted (op. cit.) that individual specimens of another taxon (*simulans*) may have repent rootstocks as long and as sparsely covered with old leaf bases as those in var. *protrusa* under unusual conditions of growth "such as, perhaps, the burying of the plant under leaf-mold or earth." We should like to caution collectors that the same phenomenon may also take place in var. *mackayii* as well, and we have some fine examples collected along roadbanks on Roe Road, Lyndon Township, Washtenaw Co., Michigan on September 11, 1955, some of the branches of which have internodes that are fully as long as those that are characteristic in var. *protrusa*.

DISCUSSION: The two taxa under consideration here may be separated by an ensemble of four morphological characters

(internodal length of rhizomes, coloration of stipes, texture of the laminae, and cutting of the leaves) and by the cytological difference in chromosome number. Although any one of the morphological characters may overlap in some cases in the two entities, as a group the morphological characters "hold together" sufficiently to make their identification dependable. The difference in chromosome number indicates that in all probability the plants do not "intergrade" in the genetical sense. (The possibility exists, however, of solitary intermediate, sterile, triploid hybrids arising, and forming clones, and we should probably seek such forms where the parents grow in proximity.) The two taxa are almost undoubtedly well isolated from each other because of their different genomes, and steady and gradual gene exchange between them is unlikely.

The diploid *protrusa* is a southern variety primarily, ranging considerably south of the known tetraploid taxa in the *Cystopteris fragilis* complex; it apparently gets no further north than southeastern New York and Minnesota. In Michigan it appears to be confined to the southern part of the state. Taxon *mackayii* extends in the east only as far south as North Carolina, Tennessee, and Missouri, and there only in the uplands, while *protrusa* ranges as far south as Alabama and Louisiana. *Mackayii* may be found northward all the way to the Canadian borders, extending at least into southern Quebec. In Michigan it ranges throughout the state, including the Upper Peninsula.

The question of the taxonomic interpretation of these two taxa is now emphasized by the recent conclusion of Löve that when different chromosome numbers are met with in morphologically distinguishable types, those types should be considered not as "intraspecific chromosome races" but as distinct species. Should "var. *protrusa*" and "var. *mackayii*" be regarded as species? Due to the sterility barrier of different chromosome numbers, such types are not classifiable, according to Löve (1955) as subspecies or varieties only, but they should be classified instead into the category of separate species. In the present case, there is no question that real morphological (and, judging from what is known of their ranges and habitat amplitudes, physiological as well) differences do exist between the taxa *protrusa* and *mackayii*. It is conceivable, therefore, that

we shall in the future be led to interpret them as distinct species, coordinate with *C. bulbifera* and *C. montana*. However, it would be premature to take this action until the whole world-wide complex is subjected to examination, and we hope that such a broad investigation will materialize.

Summary

Earlier studies of the *Cystopteris fragilis* complex led to a prediction that a diploid variety might exist. Such a diploid has now been found, with $n = 42$, in terrestrial habitats in southern Michigan. The diploid is var. *protrusa* Weatherby; but a tetraploid in the same region is var. *mackayii* Lawson. Studies of these two taxa revealed some distinctions not heretofore emphasized, and they may be separated by the following ensemble of characters: (a) internodal length of stems; (b) coloration of petioles; (c) texture of laminae; and (d) cutting of leaf-blades. Since the taxa are well isolated by their different genomes, and since they are readily distinguished morphologically, they may constitute distinct species.—UNIVERSITY OF MICHIGAN, ANN ARBOR, AND CRANBROOK INSTITUTE OF SCIENCE, BLOOMFIELD HILLS, MICHIGAN.

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